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P-Value Statistical Solutions

R Intern Assessment

Overview of the data

The dataset contains the following columns:

- sub: Subject identifier
- trt: Treatment group (Test or Reference)
- seq: Sequence number (the order of treatment)
- per: Period number (study period)
- timepoint_X: Concentration values at different timepoints (where X represents the timepoint number)

Required libraries

```
library(readxl)      # For reading excel file

library(dplyr)        # For data manipulation

library(tidyr)        # For Data reshaping

library(ggplot2)      # For data visualization

library(lme4)         # For mixed-effects models

library(lmerTest)     # For p-values in mixed-effects models

library(emmeans)      # For LS means

library(shiny)        # For build interactive web application

library(DT)           # For enhancing table interaction within Shiny app
```

Structure of the data

```
tibble [40 × 23] (S3: tbl_df/tbl/data.frame)
 $ sub : num [1:40] 1 2 3 4 5 6 7 8 9 10 ...
 $ TRT : chr [1:40] "T" "T" "T" "T" ...
 $ per : num [1:40] 1 2 2 1 2 1 2 1 2 1 ...
 $ seq : chr [1:40] "TR" "RT" "RT" "TR" ...
 $ 0 : num [1:40] 1 0 0 1 1 1 1 2 2 2 ...
 $ 0.33: num [1:40] 2 4 4 1 3 3 3 4 5 1 2 ...
 $ 1 : num [1:40] 10 7 6 8 6 9 8 8 7 10 ...
 $ 1.5 : num [1:40] 11 8 7 15 5 11 15 13 6 7 ...
 $ 2 : num [1:40] 19 16 13 12 13 17 17 10 12 ...
 $ 2.5 : num [1:40] 24 20 20 21 24 21 23 23 20 22 ...
 $ 3 : num [1:40] 25 29 20 20 23 20 28 29 29 20 ...
 $ 3.5 : num [1:40] 39 34 39 30 35 37 34 34 30 38 ...
 $ 4 : num [1:40] 38 38 34 39 38 35 39 39 32 33 ...
 $ 5 : num [1:40] 36 40 33 38 33 38 37 34 34 39 ...
 $ 6 : num [1:40] 32 31 30 33 33 31 35 30 35 31 ...
 $ 7 : num [1:40] 33 33 27 29 25 32 28 31 35 27 ...
 $ 8 : num [1:40] 24 29 29 28 24 20 22 30 30 23 ...
 $ 9 : num [1:40] 25 25 22 25 25 24 20 22 25 20 ...
 $ 12 : num [1:40] 20 19 16 17 16 15 20 17 17 17 ...
 $ 14 : num [1:40] 10 11 16 18 20 18 12 18 17 19 ...
 $ 16 : num [1:40] 2 4 5 4 4 4 2 5 2 2 ...
 $ 20 : num [1:40] 1 2 0 0 2 2 0 1 1 1 ...
 $ 24 : num [1:40] 0 1 1 0 1 0 1 0 1 0 ...
```

First Few Rows of the Dataset

```
# A tibble: 6 x 23
  sub TRT per seq `0` `0.33` `1` `1.5` `2` `2.5` `3` `3.5` `4` `5` `6` `7` `8` `9` `12`
  <dbl> <chr> <dbl> <chr> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>
1     1 T     1 TR     1     2    10    11    19    24    25    39    38    36    32    33    24    25    20
2     2 T     2 RT     0     4     7     8    16    20    29    34    38    40    31    33    29    25    19
3     3 T     2 RT     0     4     6     7    13    20    20    39    34    33    30    27    29    22    16
4     4 T     1 TR     1     1     8    15    12    21    20    30    39    38    33    29    28    25    17
5     5 T     2 RT     1     3     6     5    13    24    23    35    38    33    33    25    24    25    16
6     6 T     1 TR     1     3     9    11    17    21    20    37    35    38    31    32    20    24    15
# i 4 more variables: `14` <dbl>, `16` <dbl>, `20` <dbl>, `24` <dbl>
```

Reshaping to long format

First Few Rows of the Dataset

```
# A tibble: 760 x 6
  sub TRT per seq time concentration
  <dbl> <chr> <dbl> <chr> <dbl> <dbl>
1     1 T     1 TR     0         1
2     1 T     1 TR    0.33        2
3     1 T     1 TR     1        10
4     1 T     1 TR    1.5        11
5     1 T     1 TR     2        19
6     1 T     1 TR    2.5        24
7     1 T     1 TR     3        25
8     1 T     1 TR    3.5        39
9     1 T     1 TR     4        38
10    1 T     1 TR     5        36
# i 750 more rows
# i Use `print(n = ...)` to see more rows
```

1. Calculation of PK Parameters by treatment wise

- C_{max} (Maximum Concentration): The highest concentration of a drug in the bloodstream after administration, representing the peak concentration reached post-dose.

$$C_{\max} = \max(\text{concentration})$$

- AUC_t (Area Under the Concentration-Time Curve from 0 to the last measurable concentration): The total drug exposure over time from time 0 to the last measurable concentration using Linear trapezoidal rule.

$$AUC_t = \sum_{i=1}^{n-1} \left[\frac{C_i + C_{i+1}}{2} \times (T_{i+1} - T_i) \right]$$

Where: C_i and C_{i+1} are the concentrations at time points T_i and T_{i+1}.

- AUC_∞ (Area Under the Concentration-Time Curve from 0 to infinity): The total drug exposure from time 0 to infinity, including the AUC_t and the extrapolated area.

$$AUC_{\infty} = AUC_t + \frac{C_{\text{last}}}{k}$$

Where:

- C_{last}: Concentration at the last measurable time point.
- k: Elimination rate constant (see below).
- kel (Elimination Rate Constant): The rate at which the drug is eliminated from the body, calculated from the terminal elimination phase.

From log-linear regression: $k = |\text{slope of } (\ln(\text{concentration}) \text{ vs. time})|$

- t_{1/2} (Half-life): The time required for the drug concentration to decrease by half, related to the elimination rate constant (kel).

$$t_{1/2} = \frac{\ln(2)}{k}$$

- Tmax (Time to Reach Maximum Concentration): The time at which the drug reaches its peak concentration (C_{max}).

T_{max}=T[i] where C[i]=C_{max}

Below is the table of calculated treatment wise parameters:

	Sub	Trmt	Seq	Per	kel	Tmax	Cmax	AUCt	AUCi	t_half
1	1	T	TR	1	0.21255192	4.0	38	330.265	330.2650	3.261072
2	2	T	RT	2	0.35365864	3.5	39	349.515	352.3426	1.959933
3	3	T	RT	2	0.27409661	5.0	37	343.430	347.0783	2.528843
4	4	T	TR	1	0.04897858	5.0	40	362.595	362.5950	14.152048
5	5	T	RT	2	0.33326198	4.0	40	357.840	360.8406	2.079887
6	6	T	TR	1	0.15473034	3.5	39	332.510	332.5100	4.479711
7	7	T	RT	2	0.27546747	3.5	36	352.760	356.3902	2.516258
8	8	T	RT	2	0.53524077	4.0	39	352.095	352.0950	1.295019
9	9	T	TR	1	0.43054120	4.0	37	337.600	339.9227	1.609944
10	10	T	RT	2	0.05596638	4.0	38	350.675	350.6750	12.385064
11	11	T	RT	2	0.03515974	5.0	37	340.600	340.6000	19.714228
12	12	T	TR	1	0.33322928	5.0	38	348.680	351.6809	2.080091
13	13	T	RT	2	0.08568300	5.0	39	348.180	359.8509	8.089670
14	14	T	RT	2	0.29409781	4.0	39	338.595	338.5950	2.356859
15	15	T	TR	1	0.34853799	4.0	40	348.255	348.2550	1.988728
16	16	T	TR	1	0.35263686	4.0	39	361.260	361.2600	1.965612
17	17	T	TR	1	0.27865461	3.5	37	348.260	351.8487	2.487478
18	18	T	TR	1	0.07070578	6.0	35	348.925	363.0681	9.803261
19	19	T	RT	2	0.22468776	5.0	39	357.600	362.0506	3.084935
20	20	T	TR	1	0.34788221	5.0	39	331.930	334.8045	1.992477
21	1	R	TR	2	0.54943615	3.5	37	349.505	349.5050	1.261561
22	2	R	RT	1	0.22722743	5.0	40	338.175	342.5759	3.050456
23	3	R	RT	1	0.15987431	3.5	37	340.510	340.5100	4.335576
24	4	R	TR	2	0.06421259	5.0	38	346.425	361.9983	10.794568
25	5	R	RT	1	0.35287084	5.0	40	328.840	328.8400	1.964308
26	6	R	TR	2	0.07786180	3.5	39	344.425	357.2683	8.902275
27	7	R	RT	1	0.15429103	5.0	38	348.005	354.4863	4.492466
28	8	R	RT	1	0.43368452	5.0	36	337.180	337.1800	1.598275
29	9	R	TR	2	0.33968373	3.5	36	314.260	317.2039	2.040566
30	10	R	RT	1	0.22871752	4.0	40	338.010	338.0100	3.030582
31	11	R	RT	1	0.03920353	5.0	37	345.845	345.8450	17.680734
32	12	R	TR	2	0.35477484	5.0	37	358.755	361.5737	1.953766
33	13	R	RT	1	0.34043180	5.0	37	331.675	331.6750	2.036082
34	14	R	RT	1	0.27095612	5.0	35	344.175	347.8656	2.558153
35	15	R	TR	2	0.21763092	3.5	39	337.345	337.3450	3.184967
36	16	R	TR	2	0.03948713	4.0	40	366.180	391.5047	17.553750
37	17	R	TR	2	0.03771460	4.0	37	354.600	354.6000	18.378748
38	18	R	TR	2	0.06097599	3.5	39	363.260	363.2600	11.367542
39	19	R	RT	1	0.41989388	4.0	37	328.760	331.1416	1.650768
40	20	R	TR	2	0.04970133	4.0	40	355.095	355.0950	13.946251

2. Visualization of Test vs. Reference Ratios

Plot the subject vs. Test/Reference ratios for the following parameters and include reference lines at 0.8 and 1.25 on the line plot:

- Cmax (Maximum Concentration)
- AUCt (Area Under the Concentration-Time Curve from 0 to the last measurable concentration)
- AUCi (Area Under the Concentration-Time Curve from 0 to infinity)

Test data:

	Sub	Trmt_T	Seq_T	Per_T	kel_T	Tmax_T	Cmax_T	AUCt_T	AUCi_T	t_half_T
1	1	T	TR	1	0.21255192	4.0	38	330.265	330.2650	3.261072
2	2	T	RT	2	0.35365864	3.5	39	349.515	352.3426	1.959933
3	3	T	RT	2	0.27409661	5.0	37	343.430	347.0783	2.528843
4	4	T	TR	1	0.04897858	5.0	40	362.595	362.5950	14.152048
5	5	T	RT	2	0.33326198	4.0	40	357.840	360.8406	2.079887
6	6	T	TR	1	0.15473034	3.5	39	332.510	332.5100	4.479711
7	7	T	RT	2	0.27546747	3.5	36	352.760	356.3902	2.516258
8	8	T	RT	2	0.53524077	4.0	39	352.095	352.0950	1.295019
9	9	T	TR	1	0.43054120	4.0	37	337.600	339.9227	1.609944
10	10	T	RT	2	0.05596638	4.0	38	350.675	350.6750	12.385064
11	11	T	RT	2	0.03515974	5.0	37	340.600	340.6000	19.714228
12	12	T	TR	1	0.33322928	5.0	38	348.680	351.6809	2.080091
13	13	T	RT	2	0.08568300	5.0	39	348.180	359.8509	8.089670
14	14	T	RT	2	0.29409781	4.0	39	338.595	338.5950	2.356859
15	15	T	TR	1	0.34853799	4.0	40	348.255	348.2550	1.988728
16	16	T	TR	1	0.35263686	4.0	39	361.260	361.2600	1.965612
17	17	T	TR	1	0.27865461	3.5	37	348.260	351.8487	2.487478
18	18	T	TR	1	0.07070578	6.0	35	348.925	363.0681	9.803261
19	19	T	RT	2	0.22468776	5.0	39	357.600	362.0506	3.084935
20	20	T	TR	1	0.34788221	5.0	39	331.930	334.8045	1.992477

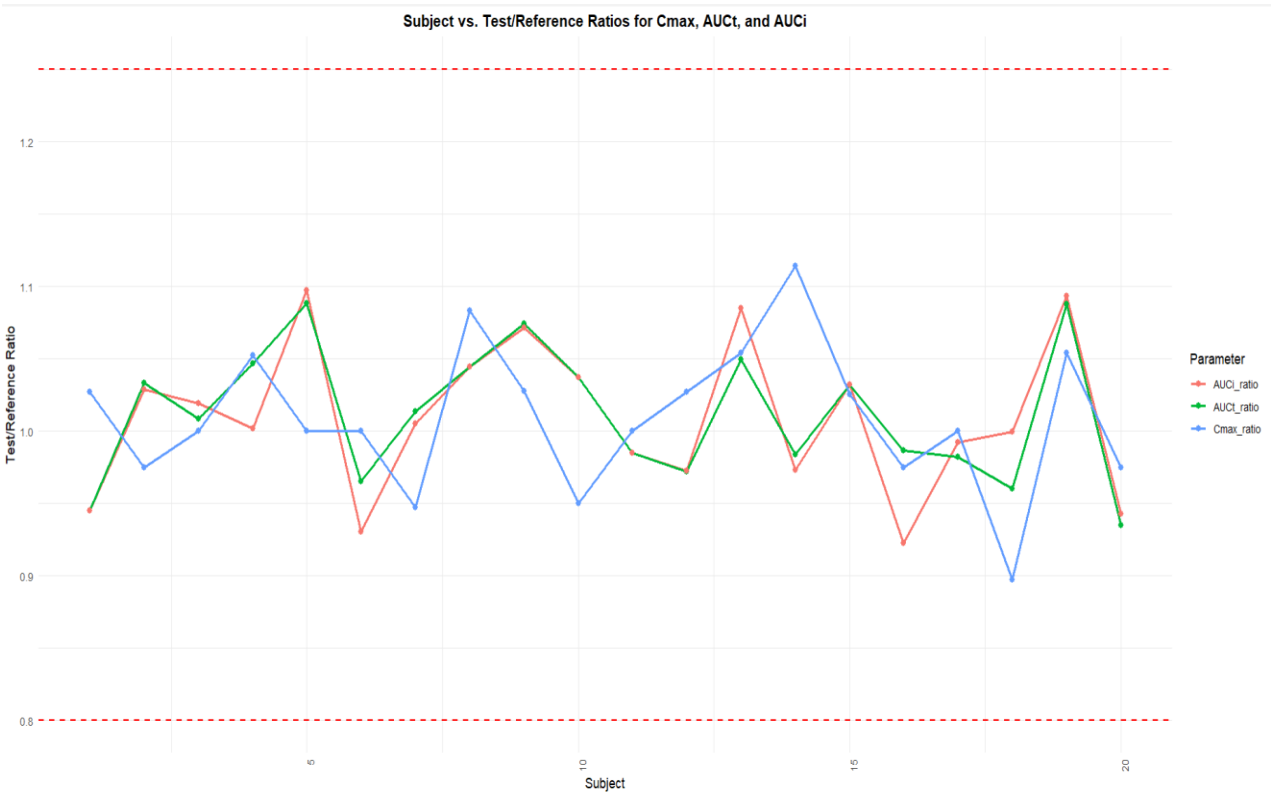
Reference data:

	Sub	Trmt_R	Seq_R	Per_R	kel_R	Tmax_R	Cmax_R	AUCt_R	AUCi_R	t_half_R
1	1	R	TR	2	0.54943615	3.5	37	349.505	349.5050	1.261561
2	2	R	RT	1	0.22722743	5.0	40	338.175	342.5759	3.050456
3	3	R	RT	1	0.15987431	3.5	37	340.510	340.5100	4.335576
4	4	R	TR	2	0.06421259	5.0	38	346.425	361.9983	10.794568
5	5	R	RT	1	0.35287084	5.0	40	328.840	328.8400	1.964308
6	6	R	TR	2	0.07786180	3.5	39	344.425	357.2683	8.902275
7	7	R	RT	1	0.15429103	5.0	38	348.005	354.4863	4.492466
8	8	R	RT	1	0.43368452	5.0	36	337.180	337.1800	1.598275
9	9	R	TR	2	0.33968373	3.5	36	314.260	317.2039	2.040566
10	10	R	RT	1	0.22871752	4.0	40	338.010	338.0100	3.030582
11	11	R	RT	1	0.03920353	5.0	37	345.845	345.8450	17.680734
12	12	R	TR	2	0.35477484	5.0	37	358.755	361.5737	1.953766
13	13	R	RT	1	0.34043180	5.0	37	331.675	331.6750	2.036082
14	14	R	RT	1	0.27095612	5.0	35	344.175	347.8656	2.558153
15	15	R	TR	2	0.21763092	3.5	39	337.345	337.3450	3.184967
16	16	R	TR	2	0.03948713	4.0	40	366.180	391.5047	17.553750
17	17	R	TR	2	0.03771460	4.0	37	354.600	354.6000	18.378748
18	18	R	TR	2	0.06097599	3.5	39	363.260	363.2600	11.367542
19	19	R	RT	1	0.41989388	4.0	37	328.760	331.1416	1.650768
20	20	R	TR	2	0.04970133	4.0	40	355.095	355.0950	13.946251

Test/Reference ratios:

	Sub	Cmax_ratio	AUCt_ratio	AUCi_ratio
1	1	1.0270270	0.9449507	0.9449507
2	2	0.9750000	1.0335329	1.0285096
3	3	1.0000000	1.0085754	1.0192897
4	4	1.0526316	1.0466768	1.0016484
5	5	1.0000000	1.0881888	1.0973137
6	6	1.0000000	0.9654061	0.9307012
7	7	0.9473684	1.0136636	1.0053710
8	8	1.0833333	1.0442345	1.0442345
9	9	1.0277778	1.0742697	1.0716219
10	10	0.9500000	1.0374693	1.0374693
11	11	1.0000000	0.9848342	0.9848342
12	12	1.0270270	0.9719168	0.9726397
13	13	1.0540541	1.0497626	1.0849504
14	14	1.1142857	0.9837873	0.9733500
15	15	1.0256410	1.0323408	1.0323408
16	16	0.9750000	0.9865640	0.9227475
17	17	1.0000000	0.9821207	0.9922410
18	18	0.8974359	0.9605379	0.9994718
19	19	1.0540541	1.0877236	1.0933409
20	20	0.9750000	0.9347639	0.9428591

Subject vs. Test/Reference ratios



Conclusion: Since all ratios are within the 0.8 to 1.25 range, the test formulation is bioequivalent to the reference formulation for Cmax, AUCt, and AUCi, ensuring consistent efficacy and safety profiles across different subjects.

3. Statistical analysis

Model the data log transformed PK parameters (C_{max}, AUC_t and AUC_i) using treatment, sequence, period and subject nested with in the sequence as fixed effects:

- Find Least square (LS) means for Treatments
- Calculate the ANOVA and find p-values

Mixed-Effects Models:

Mixed-effects models were built for each log-transformed PK parameter. The models included the following terms:

- Fixed effects: Treatment (Trmt), Sequence (Seq), and Period (Per).
- Random effect: Subject nested within Sequence (Seq:Sub).

Model equation:

$$\log_e(C_{\max}) = \beta_0 + \beta_1(\text{Trmt}) + \beta_2(\text{Seq}) + \beta_3(\text{Per}) + (1|\text{Seq:Sub})$$

LS Means for C_{max}:

Trmt	emmean	SE	df	lower.CL	upper.CL
R	3.635	0.00888	34.66	3.617	3.653
T	3.644	0.00888	34.66	3.625	3.662

Results are averaged over the levels of: Seq, Per
Degrees-of-freedom method: kenward-roger
Confidence level used: 0.95

LS Means for AUC_t:

Trmt	emmean	SE	df	lower.CL	upper.CL
R	5.839	0.00694	35.43	5.825	5.853
T	5.849	0.00694	35.43	5.835	5.863

Results are averaged over the levels of: Seq, Per
Degrees-of-freedom method: kenward-roger
Confidence level used: 0.95

LS Means for AUC_i:

Trmt	emmean	SE	df	lower.CL	upper.CL
R	5.849	0.00819	34.12	5.833	5.866
T	5.857	0.00819	34.12	5.840	5.874

Results are averaged over the levels of: Seq, Per
Degrees-of-freedom method: kenward-roger
Confidence level used: 0.95

ANOVA Results:

C_{max}

Type III Analysis of Variance Table with Satterthwaite's method						
	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Trmt	0.00065095	0.00065095	1	18	0.5135	0.4828
Seq	0.00019099	0.00019099	1	18	0.1507	0.7025
Per	0.00067542	0.00067542	1	18	0.5328	0.4748

Interpretation: The results indicate that as $p\text{-value} > 0.05$, the maximum concentration of the drug is not significantly affected by the treatment, the order of treatments, or the time period in this study.

AUCt

```
Type III Analysis of Variance Table with Satterthwaite's method
      Sum Sq   Mean Sq NumDF DenDF F value  Pr(>F)
Trmt  0.0011044 0.0011044     1    18  1.3121 0.26701
Seq   0.0006841 0.0006841     1    18  0.8128 0.37921
Per   0.0046487 0.0046487     1    18  5.5228 0.03038 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Interpretation: While the treatment and sequence of administration do not significantly affect AUCt, the time period of administration shows a significant impact. This suggests that external factors associated with different time periods (e.g., physiological or environmental conditions) might influence the drug's area under the curve.

AUCi

```
Type III Analysis of Variance Table with Satterthwaite's method
      Sum Sq   Mean Sq NumDF DenDF F value  Pr(>F)
Trmt  0.0005844 0.0005844     1    18  0.5693 0.46028
Seq   0.0013452 0.0013452     1    18  1.3104 0.26731
Per   0.0077027 0.0077027     1    18  7.5034 0.01348 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Interpretation: The treatment and sequence of administration do not significantly affect AUCi. However, a significant period effect suggests that the timing of treatment administration within the study has a measurable influence on the total drug exposure.

The End!